Amendments to the Specification:

Please replace the title of the application on page 1, line 1, with the following amended title:

POLYNUCLEOTIDES AND POLYPEPTIDES THAT CONFER INCREASED BIOMASS AND TOLERANCE TO COLD, WATER DEPRIVATION AND LOW NITROGEN TO ~~IN~~ PLANTS

Please replace the paragraph, added in the amendment of July 27, 2005, on page 1 at line 10 (before "Technical Field") with the following amended paragraph:

The claimed invention, in the field of functional genomics and the characterization of plant genes for the improvement of plants, was made by or on behalf of Mendel Biotechnology, Inc. and Monsanto Corporation as a result of activities undertaken within the scope of a joint research agreement, said agreement having been ~~executed on 10/31/1997, and~~ in effect on or before the date the claimed invention was made.

Please replace the paragraph on page 15, lines 21-24 with the following amended paragraph:

CD-ROM1 (Copy 1) is a read-only memory computer-readable compact disc and contains a copy of the Sequence Listing in ASCII text format. The Sequence Listing is named "MBI0054.ST25.txt", was created on September 16, 2003, and is 4,193 kilobytes in size. The copies of the Sequence Listing on the CD-ROM disc are hereby incorporated by reference in their entirety.

Please replace the paragraph on page 15, line 36, through page 16, line 6 with the following amended paragraph:

~~Figure 3A illustrates~~ Figures 3A-3B illustrate an example of an osmotic stress assay. The medium used in this root growth assay contained polyethylene glycol (PEG). After germination, the seedlings of a 35S::G47 overexpressing line (the eight seedlings on left of Figure 3A labeled "OE.G47--22") appeared larger and had more root growth than the four wild-type seedlings on the right. As would be predicted by the osmotic stress assay, G47 plants showed enhanced survival and drought tolerance in a soil-based drought assay, as did G2133, a paralog of G47 (see ~~Figures 10A and 10B~~ Figures 10A-10B). ~~Figure 3B also demonstrates an~~ An interesting effect of G47 overexpression is shown in Figure 3B; the 35S::G47 plants on the left and in the center of this photograph had short, thick, fleshy inflorescences with reduced apical dominance.

Please replace the paragraph on page 16, lines 15-22 with the following amended paragraph:

~~Figure 5A is a photograph of~~ Figures 5A-5D compare *Arabidopsis* 35S::G1274 and wild-type control seedlings in abiotic stress assays. When grown on low nitrogen media supplemented with sucrose plus

glutamine ~~. Seedlings~~ , seedlings of two G1274 overexpressing lines ~~are present on this plate (not distinguished), and both lines~~ (Figure 5A) contained less anthocyanin than ~~the~~ wild-type seedlings ~~seen in~~ (Figure 5B). The lack of anthocyanin production indicated that these lines were less stressed than control seedlings under the same conditions~~, a fact later confirmed in soil-based drought assays showing enhanced drought tolerance by G1274 overexpressing lines~~. G1274 ~~overexpression~~ overexpressors (Figure 5C) and wild-type (Figure 5D) ~~germination was~~ were also compared in a cold germination assay, in which the overexpressors were found to be larger and greener than the controls.

Please replace the paragraph on page 16, line 29 through page 17, line 2 with the following amended paragraph:

Figures ~~7A and 7B~~ 7A-7D compare growth of *Arabidopsis* G1792 overexpressing ~~*Arabidopsis* seedling growth~~ seedlings and wild-type controls on a single plate (two sectors of the same plate) ~~with medium containing 3% sucrose medium lacking nitrogen~~, five days after planting. ~~The~~ On a medium lacking nitrogen and containing 3% sucrose, the 35S::G1792 lines seen in Figure 7A generally showed greater cotyledon expansion and root growth than the wild-type seedlings in Figure 7B. Figure 7C is a photograph of a single plate showing a G1792 overexpressing line (labeled G1792-12; on left) and wild-type plants (on right) five days after inoculation with *Botrytis cinerea*, showing the chlorosis and hyphal growth in the latter control plants but not in the former overexpressors. Similar results were obtained five days after inoculation with *Erysiphe orontii* (not shown) and with *Fusarium oxysporum*, as seen in Figure 7D, with control plants on the right showing chlorosis, and G1792 overexpressors on the left appearing to be free of the adverse effects of infection.

Please replace the paragraph on page 17, lines 3-11 with the following amended paragraph:

Figures 8A-8C show results obtained with G2999 overexpressing *Arabidopsis* plants in high salt assays. Figure 8A illustrates the results of root growth assays with G2999 overexpressing seedlings and controls in a high sodium chloride medium. The eight 35S::G2999 *Arabidopsis* seedlings on the left were larger, greener, and had more root growth than the four control seedlings on the right. Another member of the G2999 clade, G2998, also showed a salt tolerance phenotype and performed similarly in the plate-based salt stress assay seen Figure 8B. In the latter assay 35S::G2998 seedlings appeared large and green, whereas wild-type seedlings in the control assay plate shown in Figure 8C were small and had not yet expanded their cotyledons. As is noted below, high sodium chloride growth assays often are used to indicate osmotic stress tolerance such as drought tolerance, which was subsequently confirmed with soil-based assays conducted with G2999-overexpressing plants.

Please replace the paragraph on page 17, lines 12-16 with the following amended paragraph:

~~Figure 9A shows~~ Figures 9A-9B compare the effects of a heat assay (Figure 9A) and a high salt assay (Figure 9B) on *Arabidopsis* wild-type and G3086-overexpressing plants. Generally, the overexpressors on the left of Figure 9A were larger, paler, and bolted earlier than the wild type plants seen on the right in this plate. The same G3086 overexpressing lines, as exemplified by the eight seedlings on the left of Figure 9B, were also found to be larger, greener, and had more root growth in a high salt root growth assay than control plants, including the four on the right in Figure 9B.

Please replace the paragraph on page 17, lines 17-22 with the following amended paragraph:

~~Figures 10A and 10B~~ Figures 10A-10B compare the recovery from a drought treatment in two lines of G2133 overexpressing *Arabidopsis* plants and wild-type controls. Figure 10A shows plants of 35S::G2133 line 5 (left) and control plants (right). Figure 10B shows plants of 35S::G2133 line 3 (left) and control plants (right). Each pot contained several plants grown under 24 hours light. All were deprived of water for eight days, and are shown after re-watering. All of the plants of the G2133 overexpressor lines recovered, and all of the control plants were either dead or severely and adversely affected by the drought treatment.

Please replace the paragraph on page 25, line 30, through page 26, line 4 with the following amended paragraph:

With regard to polynucleotide variants, differences between presently disclosed polynucleotides and polynucleotide variants are limited so that the nucleotide sequences of the former and the latter are closely similar overall and, in many regions, identical. Due to the degeneracy of the genetic code, differences between the former and latter nucleotide sequences [[o]] may be silent (i.e., the amino acids encoded by the polynucleotide are the same, and the variant polynucleotide sequence encodes the same amino acid sequence as the presently disclosed polynucleotide. Variant nucleotide sequences may encode different amino acid sequences, in which case such nucleotide differences will result in amino acid substitutions, additions, deletions, insertions, truncations or fusions with respect to the similar disclosed polynucleotide sequences. These variations result in polynucleotide variants encoding polypeptides that share at least one functional characteristic. The degeneracy of the genetic code also dictates that many different variant polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

Please replace the paragraph on page 38, lines 7-11 with the following amended paragraph:

(4) Sequences of three *Arabidopsis* GAMYB-like genes were obtained on the basis of sequence similarity to GAMYB genes from barley, rice, and *L. temulentum*. These three ~~*Arabadopsis*~~ *Arabidopsis* genes were determined to encode transcription factors (AtMYB33, AtMYB65, and AtMYB101) and could

substitute for a barley GAMYB and control alpha-amylase expression (Gocal et al. (2001) *Plant Physiol.* 127: 1682-1693).

Please replace the paragraph on page 56, lines 36 to page 57, line 19 with the following amended paragraph:

A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a ~~phentoype~~ phenotype or trait of interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream genes that are subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homolog of the invention is expressed in a host cell, e.g., a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (such as binding sites on DNA sequences) involved in the regulation of a downstream target. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA-binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al. (1999) *Nature Biotechnol.* 17: 573-577).

Please replace the column headings on page 81, Table 5, first row, with the following amended column headings (the last two column headings were reversed):

Polypeptide SEQ ID NO:	GID No.	~~Family~~ Conserved Domains in Amino Acid Coordinates	~~Conserved Domains in Amino Acid Coordinates~~ Family

Please replace the paragraph on page 112, lines 15-16 with the following amended paragraph (note space after "G961" in line 15):

Transcription factor genes that alter seed shape, including G652, G916, G961 and their equivalogs may have both ornamental applications and improve or broaden the appeal of seed products.

Please replace the paragraph on page 116, line 17 through page 117, line 2 with the following amended paragraph:

Suppression of endogenous transcription factor gene expression can also be achieved using RNA interference , or RNAi. RNAi is a post-transcriptional, targeted gene-silencing technique that uses double-stranded RNA (dsRNA) to incite degradation of messenger RNA (mRNA) containing the same sequence as the dsRNA (Constans, (2002) *The Scientist* 16:36). Small interfering RNAs, or siRNAs are produced in at least two steps: an endogenous ribonuclease cleaves longer dsRNA into shorter, 21-23 nucleotide-long RNAs. The siRNA segments then mediate the degradation of the target mRNA (Zamore, (2001) *Nature Struct. Biol.*, 8:746-50). RNAi has been used for gene function determination in a manner similar to antisense oligonucleotides (Constans, (2002) *The Scientist* 16:36). Expression vectors that continually express siRNAs in transiently and stably transfected cells have been engineered to express small hairpin RNAs (shRNAs), which get processed in vivo into siRNAs-like molecules capable of carrying out gene-specific silencing (Brummelkamp et al., (2002) *Science* 296:550-553, and Paddison, et al. (2002) *Genes & Dev.* 16:948-958). Post-transcriptional gene silencing by double-stranded RNA is discussed in further detail by Hammond et al. (2001) *Nature Rev Gen* 2: 110-119, Fire et al. (1998) *Nature* 391: 806-811 and Timmons and Fire (1998) *Nature* 395: 854. Vectors in which RNA encoded by a transcription factor or transcription factor homolog cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in US Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

Please replace the paragraph on page 117, lines 3-15 with the following amended paragraph:

Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to suppress expression of an endogenous transcription factor, thereby reducing or eliminating its activity and modifying one or more traits. Methods for producing such constructs are described in US Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a

plant trait can be modified by gene silencing using ~~double-strand~~ double-stranded RNA (Sharp (1999) *Genes and Development* 13: 139-141). Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homolog gene. Plants containing a single transgene insertion event at the desired gene can be crossed to generate homozygous plants for the mutation. Such methods are well known to those of skill in the art (See for example Koncz et al. (1992) Methods in *Arabidopsis* Research, World Scientific Publishing Co. Pte. Ltd., River Edge, NJ).

Please replace the paragraph on page 118, lines 8-15 with the following amended paragraph:

The plant can be any higher plant, including gymnosperms, monocotyledonous and ~~dicotyledenous~~ dicotyledonous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean, clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcurbitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al., eds., (1984) Handbook of Plant Cell Culture –Crop Species, Macmillan Publ. Co., New York, NY; Shimamoto et al. (1989) *Nature* 338: 274-276; Fromm et al. (1990) *Bio/Technol.* 8: 833-839; and Vasil et al. (1990) *Bio/Technol.* 8: 429-434.

Please replace the paragraph on page 120, lines 32-37 with the following amended paragraph:

The methods of this invention can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may be implemented on a single computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

Please replace the paragraph on page 362, line 33 through page 363, line 5 with the following amended paragraph:

After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of *Agrobacterium tumefaciens* cells for transformation ~~were~~ was made as described by Nagel et al. (1990) *FEMS Microbiol Letts.* 67: 325-328. *Agrobacterium* strain ABI was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance over 1 cm at 600 nm (A_{600}) of 0.5 – 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 µl chilled buffer (1 mM

HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125 µl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 µl and 750 µl, respectively. Resuspended cells were then distributed into 40 µl aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

Please replace the paragraph on page 371, lines 24-29 with the following amended paragraph:

Salt stress assays are intended to find genes that confer better germination, seedling vigor or growth in high salt. Evaporation from the soil surface causes upward water movement and salt accumulation in the upper soil layer where the seeds are placed. Thus, germination normally takes place at a salt concentration much higher than the mean salt concentration [[of]] in the whole soil profile. Plants differ in their tolerance to NaCl depending on their stage of development, therefore seed germination, seedling vigor, and plant growth responses are evaluated.

Please replace the paragraph on page 392, lines 21-24 with the following amended paragraph (note space after "G581" in line 22):

The function of G581 was first studied by knockout analysis. Homozygous plants containing a T-DNA insertion within the first half of the G581_coding region displayed wild-type morphology at all developmental stages. Furthermore, G581 knockout mutant plants behaved similarly to wild type in all physiological and biochemical assays performed.

Please replace the paragraph on page 427, lines 4-7 with the following amended paragraph (note space after "T1" in line 6):

G1792 overexpressing plants showed several mild morphological alterations: leaves were dark green and shiny, and plants bolted, subsequently senesced, slightly later than wild-type controls. Among the T1_ plants, additional morphological variation (not reproduced later in the T2 plants) was observed: many showed reductions in size as well as aberrations in leaf shape, phyllotaxy, and flower development.

Please replace the paragraph on page 456, lines 20-22 with the following amended paragraph (note space after "G2661" on line 20):

The sequence of G2661_was obtained from the *Arabidopsis* genome sequencing project, GenBank accession number AL161746, nid=7327833, based on its sequence similarity within the conserved domain to other bHLH related proteins in *Arabidopsis*.

Please replace the paragraph on page 485, lines 9-20 with the following amended paragraph:

The "one-hybrid" strategy (Li and Herskowitz (1993) *Science* 262: 1870-1874) is used to screen for plant cDNA clones encoding a polypeptide comprising a transcription factor DNA binding domain, a

conserved domain. In brief, yeast strains are constructed that contain a lacZ reporter gene with either wild-type or mutant transcription factor binding promoter element sequences in place of the normal UAS (upstream activator sequence) of the ~~GALL~~ GAL4 promoter. Yeast reporter strains are constructed that carry transcription factor binding promoter element sequences as UAS elements are operably linked upstream (5') of a lacZ reporter gene with a minimal ~~GAL1~~ GAL4 promoter. The strains are transformed with a plant expression library that contains random cDNA inserts fused to the GAL4 activation domain (GAL4-ACT) and screened for blue colony formation on X-gal-treated filters (X-gal: 5-bromo-4-chloro-3-indolyl-ß-D-galactoside; Invitrogen Corporation, Carlsbad CA). Alternatively, the strains are transformed with a cDNA polynucleotide encoding a known transcription factor DNA binding domain polypeptide sequence.

Please replace the paragraph on page 485, lines 21-29 with the following amended paragraph:

Yeast strains carrying these reporter constructs produce low levels of beta-galactosidase and form white colonies on filters containing X-gal. The reporter strains carrying wild-type transcription factor binding promoter element sequences are transformed with a polynucleotide that encodes a polypeptide comprising a plant transcription factor DNA binding domain operably linked to the acidic activator domain of the yeast GAL4 transcription factor, "GAL4-ACT". The clones that contain a polynucleotide encoding a transcription factor DNA binding domain operably linked to ~~GLA4-ACT~~ GAL4-ACT can bind upstream of the lacZ reporter genes carrying the wild-type transcription factor binding promoter element sequence, activate transcription of the lacZ gene and result in yeast forming blue colonies on X-gal-treated filters.

Please replace the paragraph on page 489, lines 16-19 with the following amended paragraph:

Similarly, the amino acid sequences of the three CBF polypeptides range from 84 to 86% identity. An alignment of the three amino ~~acidic~~ acid sequences reveals that most of the differences in amino acid sequence occur in the acidic C-terminal half of the polypeptide. This region of CBF1 serves as an activation domain in both yeast and *Arabidopsis* (not shown).

Please replace the Abstract on page 500, lines 4-8 with the following amended paragraph:

The invention relates to plant transcription factor polypeptides, polynucleotides that encode them, homologs from a variety of plant species, and methods of using the polynucleotides and polypeptides to produce transgenic plants having advantageous properties compared to a reference plant. ~~Sequence information related to these polynucleotides and polypeptides can also be used in bioinformatic search methods and is also disclosed.~~ These properties include increased biomass and increased tolerance to cold, water deprivation, and low nitrogen conditions.